IMAC - Immobilized Metal-Affinity Chromatography

Aim

To purify a protein fused with a His_6-tag using IMAC.

Procedure

Preparations

1. Harvest cells and spin at 5000 g for 10 min, 4 °C
2. Resuspend in 10 ml Buffer A
3. Lyse cells by French press (1000 psi, 3 rounds) or using sonication.
4. Spin at 17000 x g for 10 min, 4 °C and collect supernatant

Method

1. Let the storage buffer (the buffer already in the pre-packed column) drain from the resin by gravity flow. Pulse column with ... ml (3 CV) dH_2O.
2. Add ... ml (2 CV) of Buffer C.
3. Equilibrate the column with ... ml (5 CV) of Buffer A. Allow the buffer to drain from the resin.
4. Add the prepared protein extract onto the resin.
5. Wash resin with ... ml (3 CV) of Buffer A.
6. Elute His-tagged proteins from the resin with ... ml (0.5 CV) Buffer C. Repeat this step 5 times. Collect each fraction in a separate tube.
7. Re-equilibrate the column by following steps 2 and 3.
8. Add ... ml (2 CV) 20 % EtOH until it fills the column. Seal the column.

Buffer A: 20 mM Tris (pH 8.5)
Buffer B: 20 mM Tris, 250 mM Imidazole

Note!

CV means column volume and is calculated by calculating the volume of the resin which is the volume of a cylinder.
The IMAC columns have to be filled with liquid at all times to prevent them from drying out.
Mark all the collection tubes with the correct fraction names; protein extract, flow-through, wash, elution...
In step 4: the flow-through can be collected in a tube and if desired, re-applied into the column for maximum binding!

Sources

Protocol revised from the course BB1105, KTH Royal Institute of Technology.